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RESEARCH ON COLD STERILIZATION WITH FORMALIN VAPORS

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16. Abstract The sterilization efficacy of formalin vaporized in a 20 ℓ sterilizer operated at 70° is compared with that of ethylene oxide, using spores of B. subtilis. Neither produces good results with tap water with spore counts higher than 10^2. The two agents are roughly equivalent in killing spores in alcohol/blood specimens; formalin is superior for spores in physiological saline. However, spores in cloth pads saturated with blood and dried are more resistant to formalin. The author proposes the latter test be used for formalin sterilization apparatus.

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RESEARCH ON COLD STERILIZATION WITH FORMALIN VAPORS

Wolfgang Adam

Cold sterilization claims a place in modern medicine. The hope that the responsible industry would replace thermally unstable plastics with thermally stable materials has not materialized. We are thus forced to continue dealing with the problems of cold sterilization. /477*

The methods available so far are not yet satisfactory, however.

The use of radiation is restricted to those materials which exhibit no changes in their properties under the exposure to energy levels required. Sterilization with ethylene oxide, which was initially undertaken with great zeal, is hampered by the problem of ventilation time, since the gas apparently does not just deposit on the surfaces of the sterile material but dissolves in parts of it and is liberated again only slowly. This means that many materials can only be used several days or weeks after sterilization. An additional disadvantage of ethylene oxide is its high explosion hazard and toxicity.

For this reason, formaldehyde or mixtures of water vapor and formaldehyde have already been used for a relatively long time in England and Scandinavia as a means of cold sterilization [1, 2]. /478

We, too, had performed such experiments several years ago, but they were doomed to failure as long as the killing of spores in compost soil was considered the criterion for adequate sterilization. Only after it was realized that other biological

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* Numbers in the margin indicate pagination in the foreign text.

test objects than spore-bearing soil samples must be used as a scale for evaluating the performance of apparatus for gas sterilization was the path open for the development of a sterilization procedure with formalin.

We do have no generally acknowledged method for testing gas sterilizers, so the sterilization of certain test objects could be set as the goal for the development of a formalin method, but it seems reasonable to conclude that the method would exhibit adequate performance if it were the same as that in the ethylene oxide method.

In the method which we have developed, the chamber of the preheated sterilizer is loaded and evacuated; a certain quantity of dilute formalin solution, which is drawn up by a dosing device, is placed on the bottom. Operating temperature is 70°.

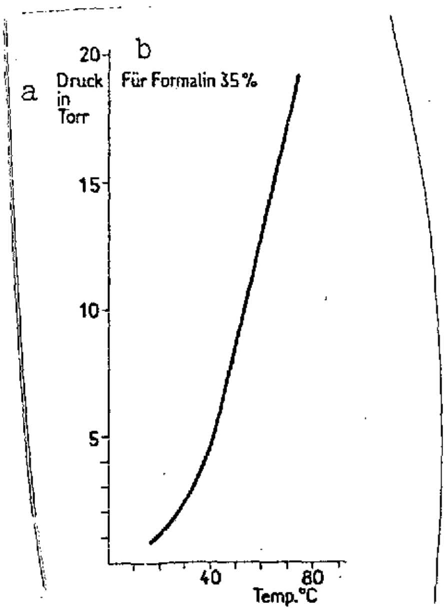

Fig. 1. Vapor pressure curve.

Key: a. Pressure in torr; b. For 35% formalin

The formalin vaporizes instantaneously in the vacuum; pressure in the chamber rises to about the water vapor pressure corresponding to that temperature.

Since the vapor pressure of formaldehyde is extremely low, it is not appreciable here.

After the programmed exposure time, the chamber is re-evacuated, the excess formalin being vaporized in the chamber. Pressure in the chamber remains in the vicinity of the corresponding water vapor pressure during this process until the formalin is completely vaporized; it then drops further.

The chamber is then flushed with filtered air, re-evacuated, and re-flushed.

If pump capacity and the evacuation and ventilation times are adequate, the formalin or formaldehyde can be removed to the extent that its odor is no longer perceivable.

Since we wished to make a comparison between the newly developed method and ethylene oxide sterilization, we first had to check whether the specimens which we used for the evaluation of ethylene oxide methods could also be sterilized by the formalin method.

We used spores of B. subtilis, as generally described in the international literature, as test bacteria for ethylene oxide sterilization.

They are cultured on a medium described by Beeby and Whitehouse; the cultures, about 90% sporulated, are flushed off with distilled water, washed three times with distilled water and once with 96% ethyl alcohol, and are then taken up in 96% alcohol. The required quantities are taken from this stock emulsion and adjusted to a spore count of 10^7/ml [3].

A given quantity of defibrinated sheep's blood is placed in a shaking bottle and is treated with 10 parts alcoholic spore suspension, with agitation. The blood flocculates in fine particles. We place 0.1 ml of the spore-blood mixture in test tubes about 100 mm long and 10 mm in diameter. The tubes are sealed with cellulose stoppers and dried at 50° in the incubator for 24 hours.

We had previously used similar specimens in which the spore /480
emulsion had been mixed with equal parts of physiological saline solution. It has been found, however, that the requirement for

TABLE 1. (THE FIGURES GIVE THE NUMBER OF POSITIVE SPECIMENS OUT OF SIX IN EACH CASE)

	Ethylene oxide							*Formalin*									
Minutes	5	10	15	20	30	45	60	5	10	15	20	30	45	60	75	90	120
Spores of <u>B. subtilis</u> in																	
Tap water 10^6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	-	6
Tap water 10^4	6	6	5	2	2	3	3	6	6	4	5	2	2	3	4	-	4
Tap water 10^2	5	5	0	1	1	0	1	6	6	3	3	2	3	2	2	-	2
Alcoholic 10^6	6	5	1	0	0	0	-	6	1	0	0	0	-	-	-	-	-
Blood-soaked pads 10^6	6	6	2	1	0	0	-	6	6	6	6	6	2	3	0	0	0
blood Na Cl 10^6	6	6	2	0	0	0	-	6	0	0	0	-	-	-	-	-	-

sterilization of the saline/spores specimens had been set too high [5, 6].

Specimens in which tap water was used in place of the saline solution could be sterilized only if the spore count was less than 10/specimen.

We had to conclude in experiments with formalin disinfection in the disinfection apparatus that spores which were dried to embroidery thread or drill pads with blood could be killed with formalin vapors only with relative difficulty.

In ethylene oxide sterilization, specimens of this type cause no particular problems.

For our comparative studies we therefore still used drill pads, 1 cm^2 in size, which were saturated with an emulsion of subtilis spores in defibrinated blood and dried. The spore count was about 10^6/specimen in each case. The pads were exposed to the vapors in stoppered test tubes.

Glucose broth was used for reculturing in the case of specimens treated with ethylene oxide and histidine broth for formalin specimens.

In the case of formalin specimens, we placed about 0.2 ml of a 2% sodium sulfide solution in the test tubes before adding the medium and allowed the solution to act for 20 min.

Incubation took 14 days at 37°C.

A cartridge-operated apparatus with a usable space of about 20 ℓ which worked by the vacuum method was used as the experimental equipment for ethylene oxide sterilization. For the formalin trials we used a converted gas sterilizer, likewise with a usable space of about 20 ℓ.

The exposure times employed and the results of the trials can be found in the accompanying table. /481

It should be noted with regard to sterilization times that even after the sterilization time is over in the formalin method, i.e. after the beginning of evacuation of the formalin vapors, it still produces an action until the residue of formalin has been evaporated. This lasted about 20 min in our experimental system.

If we look over the results, we are first struck by the fact that sterilization cannot be achieved with either method in the specimens to which tap water has been added if the spore counts are higher than 10^2.

The larger the quantity of spores contained in the specimens, the higher the probability obviously is that individual spores become encrusted in minerals which protect these spores from being attacked by the gases.

In the alcohol/blood specimens, the effect of the formalin method is equivalent to that of ethylene oxide sterilization.

The saline/spore specimens possess the same resistance to ethylene oxide as the alcohol/blood specimens; they are more sensitive in the formalin method.

The situation is reversed in the case of pads saturated in blood containing B. subtilis. They are sterilized with ethylene oxide in about the same time as the alcohol/blood specimens, but are considerably more resistant to formalin.

Our

Our experiments thus indicate that a degree of sterilization like that with ethylene oxide can be achieved with formalin vapors at 70°C.

They also show that it is not permissible just to use the same type of specimen as test material for sterilization with different gases.

I would suggest the blood/spore pads described for the testing of formalin apparatus.

Discussion Remarks

The designation "cold sterilization" does not apply here. We speak of hot-air, steam and radiation sterilization and use these terms to describe the entity which has a sterilizing effect, namely hot air, steam, or high-energy radiation. It could logically be concluded from the designation "cold sterilization" /482
that cold is involved in the sterilization process. The ethylene oxide method, which is included among the so-called cold sterilization methods, operates at temperatures of 50 to 60°C; Adam's formalin method, just reported, as high as 70°C. Temperatures of 50 to 70°C could hardly be perceived as cold.

The "cold sterilization methods" are chemical sterilization methods, which should be characterized in terms of the concentration of the sterilizing agent, exposure time and temperature. They differ from chemical disinfection methods merely in their broader action. Chemical sterilization methods must be bactericidal, sporicidal and virucidal in action.

Luckily it has not become conventional to refer to the chemical disinfection methods as "cold disinfection methods." (Heicken (Berlin))

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